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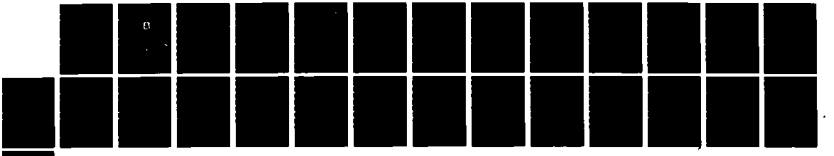

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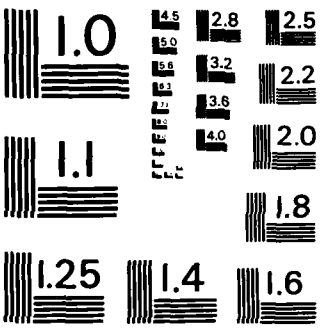

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FOREIGN TECHNOLOGY DIVISION

THEORETICAL PREREQUISITES FOR THE POSSIBLE USE OF BACTERIA WHICH SPLIT ORGANOPHOSPHATES IN ORDER TO INCREASE THE YIELD OF NUTRIENT YEAST AND ITS NITROGEN- AND PHOSPHOROUS CONTENT

by

D.L. Shamis

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EDITED TRANSLATION

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Block	Italic	Transliteration	Block	Italic	Transliteration
А а	*А а*	A, a	Р р	*Р р*	R, r
Б б	*Б б*	B, b	С с	*С с*	S, s
В в	*В в*	V, v	Т т	*Т т*	T, t
Г г	*Г г*	G, g	У у	*У у*	U, u
Д д	*Д д*	D, d	Ф ф	*Ф ф*	F, f
Е е	*Е е*	Ye, ye; E, e*	Х х	*Х х*	Kh, kh
Ж ж	*Ж ж*	Zh, zh	Ц ц	*Ц ц*	Ts, ts
З з	*З з*	Z, z	Ч ч	*Ч ч*	Ch, ch
И и	*И и*	I, i	Ш ш	*Ш ш*	Sh, sh
Й й	*Й й*	Y, y	Щ щ	*Щ щ*	Shch, shch
К к	*К к*	K, k	Ъ ъ	*Ъ ъ*	"
Л л	*Л л*	L, l	Ы ы	*Ы ы*	Y, y
М м	*М м*	M, m	Ь ь	*Ь ь*	'
Н н	*Н н*	N, n	Э э	*Э э*	E, e
О о	*О о*	O, o	Ю ю	*Ю ю*	Yu, yu
П п	*П п*	P, p	Я я	*Я я*	Ya, ya

*ye initially, after vowels, and after ъ, ь; e elsewhere.
When written as ё in Russian, transliterate as yë or ë.

RUSSIAN AND ENGLISH TRIGONOMETRIC FUNCTIONS

Russian	English	Russian	English	Russian	English
sin	sin	sh	sinh	arc sh	$\sinh^{-1}$
cos	cos	ch	cosh	arc ch	$\cosh^{-1}$
tg	tan	th	tanh	arc th	$\tanh^{-1}$
ctg	cot	cth	coth	arc cth	$\coth^{-1}$
sec	sec	sch	sech	arc sch	sech^{-1}
cosec	csc	csch	csch	arc csch	csch^{-1}

Russian	English
rot	curl
lg	log

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THEORETICAL PREREQUISITES FOR THE POSSIBLE USE OF BACTERIA WHICH SPLIT ORGANOPHOSPHATES IN ORDER TO INCREASE THE YIELD OF NUTRIENT YEAST AND ITS NITROGEN AND PHOSPHOROUS CONTENT

D. L. Shamis

In discussing the question of the maximum industrial yield of yeast in his book "Yeast Technology", John Wait (1957) starts from the assumption that, by analogy with alcoholic fermentation, 1/3 of the carbon of the usable carbohydrates of the medium is constantly converted to carbon dioxide. The remaining carbon then may be converted into yeast biomass. Wait's ideas in this respect were based on the Gay-Lussac equations:

$$\underset{(180)}{C_6H_{12}O_6} = 2\,\underset{(92)}{C_2H_5OH} + 2\,\underset{(88)}{CO_2}$$

and a theory which allows for the conversion of alcohol into cellular carbon compounds: carbohydrates, proteins, and others. 92 parts of alcohol or 48 parts of carbon obtained from 180 parts of hexose are used for the formation of these compounds. With an average carbon content of 47%, obtained from 180 parts of hexose, in the yeast dry matter, 48 parts of carbon should form $\frac{48 \cdot 100}{47} = 102.1$ parts of yeast dry matter. Based on this calculation, from 100 parts of hexose we should obtain 56.7 parts of yeast dry matter, which in the case of a yeast dry matter content of 27% should correspond to a yeast yield equal to 210% in sugar. Wait emphasizes that never in his long experience did he ever observe a yeast yield that exceeded the calculated value. Cases of a yeast

yield above the calculated value always were connected with the presence of other carbon-containing compounds easily assimilated by the yeast in the raw material in addition to the reactant.

In the preface to the Russian edition of J. Wait's book,"Yeast Technology", Prof. Veselov disagrees with the author's idea that, irrespective of the yeast yield in the conditions of abundant aeration characteristic of commercial yeast production, there should be a constant conversion of 1/3 of the carbon of the carbohydrates used into carbon dioxide. Veselov supports his objections with the fact that a significant amount of the carbon dioxide formed in the case of the aerobic growing of yeast is heterotrophically fixed by the yeast, and with ammonium salts participates in the biosynthesis of complex substances, for example alanine and others. Veselov states that heterotrophic carbon dioxide fixation is a process which yeasts actually perform, and in this case, the amount of carbon dioxide split off will not correspond to 1/3 of the carbon of the carbohydrates consumed, but always will be less. Heterotrophic carbon dioxide fixation, as Shaposhnikov (1960) noted, has been established for many representatives of the microbe world. The hypothesis that it directly participates in the synthesis of substances was first expressed by Lebedev at the beginning of this century. Wood and Werkman (1936, 1938) succeeded in proving this hypothesis by detecting the formation of succinic acid in a culture of propionic acid bacteria in glycerine in the presence of carbonates. The formation of succinic acid is based on the condensation of C_1 and a three-carbon chain, according to the reaction named after Wood and Werkman, the actual nature of which they showed for the formation of oxalic acid by means of the condensation of pyruvic acid and carbon dioxide. They also reported finding a similar phenomenon for propionic acid (1956). In the studies by Aje and Werkman (1948, 1954) with Escherichia coli, it was found that the absence of CO_2 in the air sharply reduces the development of bacteria. No small contribution to the analysis of the question of the role of heterotrophic assimilation in the metabolism of microorganisms was made by Shaposhnikov's colleagues and students - Ilyaletdinov (1955), Kupletskaya (1955) and others.

Thus heterotrophic carbon dioxide fixation for the biosynthesis of cellular compounds and other needs of the organism proved to be a property characteristic not only of yeasts, but also of many other physiological groups of microorganisms.

From the studies by Fisher (1958) and the experience of yeast growers, it is known that the yield of nutrient yeast usually is significantly less than the theoretically possible level, and amounts to 40-45% of the reactant used.

The problem of increasing the yield and improving the quality of nutrient yeast has occupied many investigators. They have studied tne physical and chemical conditions, the salt regime, the preparation techniques, and procedures for purifying and improving the quality of nutrient media from different types of inedible raw material used for raising nutrient yeast. In addition, highly productive, profitable strains of nutrient yeast have been selected. Many of the findings of these studies have been implemented in practice and have been of great use. However, even today the practical yield of yeast is significantly less than the theoretically possible level. Certain physiological aspects of yeast metabolism which determine the quality of this metabolism have not been thoroughly explained. All this lays a heavy burden on production and increases the cost of nutrient yeast, which, as is well-known, provides a significant amount of the proteins and vitamins of the growing animal husbandry in this country.

In accordance with that which is stated above, it is natural that many scientists and yeast growers are interested both in studying the nature of, and in finding the reason for, the discrepancy between the actual yeast yield and the theoretically possible level. One of these attempts to explain the cause of the reduced yeast yield is the summary of long-term studies in this area by Prof. Malkov (1961) et al. reported to the Conference of the Leningrad Section of the All-Union Microbiological Society, together with other scientific-technical societies.

Supporting Wait's ideas, Malkov sees one of the causes for the incomplete yeast yield to be an extreme expenditure, more than 1/3, of the carbon of the carbohydrates used for respiration. As a result of this uneconomical, in the author's opinion, consumption of sugar for respiration, less than 2/3 of it remains for biomass synthesis, and the yeast yield is correspondingly lowered. In order to prevent this undesirable phenomenon, Malkov, in his experiments, partially inhibited yeast respiration by keeping the yeast in concentrated organophosphate before innoculation. In view of the importance of the problem studied by Malkov, we shall present a table from his study.

Increasing the nutritional value of yeast by treating it with organophosphate.

(a) Условия обработки фосфатом	(b) Динамика, в % по отношению к контролю, принятому за 100			(c) Поглощено CO_2 дрожжами
	(d) Выход дрожжей	(e) Содержание в дрожжах		
		(f) витамина B_2	P_7	
(g) Обработка дрожжей фосфатом (P-1,08%)	115,7	213,5	228,0	90,6
(h) (i) Фосфат (P-1,08%) добавлен к среде (j)	100	100	100	100

Key: (a) Phosphate treatment conditions; (b) Dynamics in % in relation to the control taken as 100; (c) CO_2 absorbed by yeast; (d) Yeast yield; (e) Content in yeast; (f) Vitamin B_2; (g) Treatment of yeast; (h) with phosphate (P-1.08%); (i) phosphate (P-1.08%); (j) added to medium.

From the data in the table it is evident that, as a result of the aftereffect of keeping yeast in concentrated organophosphate before innoculation, the oxygen absorption was reduced by 10%, the yeast yield was increased by 15%, and the Vitamin B_2 and acid-labile phosphate P_7 content of the yeast increased by more than two times.

The results obtained by Malkov are very indicative. Bearing in mind the data in the literature on heterotrophic CO_2 fixation, he sensibly began to calculate the degree of yeast respiration inhibition only according to the amount of absorbed oxygen. However, developing Wait's idea, Malkov considers that the oxygen saved as

a result of the partial inhibition of respiration must be used for biosynthetic processes, which leads to an increase in the yeast yield. An increase in the yeast yield of 15% actually was achieved in his experiments. However, there is still no answer as to how to reconcile the strangulation of the respiratory process - the basic energy supplier for endothermic biosynthetic processes - which takes place in the case of yeast inhibition with the increase in yeast yield. In this regard, it is also difficult to reconcile Malkov's data with the study of O. Meyrhof (1925), who revealed the oxygen saving effect on sugar consumption in the case of the respiration of yeast of the genus Torula. In these experiments, conducted with aeration of the medium and without it, it was shown that three and more times less glucose is consumed (in mg per 1 mg of cells per 1 h) in the case of aeration. The carbon source for this purpose may be the carbon reserves in the cell formed as the result of endogenous exchange. After being held in concentrated organophosphate the yeast evidently absorbs the total amount of oxygen, not including the amount which it absorbed for the assimilation of the adsorbed phosphorous. This is indicated by the sharp increase in the P_7 content noted by Malkov.

Thus, in our view, the inhibition of respiration was not the main reason for the increase in yeast yield but the energy of accumulation in polyphosphates (P_7) synthesized as the result of the assimilation of the adsorbed phosphorous. It should be mentioned that the author himself remarks on this, referring to the increase in P_7, but connects the enrichment of the yeast with the "energy-rich" phosphates only with the prospects of improving their nutritional value and not with an increase in yield.

It must be mentioned that there is significant theoretical and practical interest in seeking out ways to control respiration processes and biosynthesis.

Undoubtedly, such mechanisms must exist in the complex of regulatory mechanisms of the yeast cell. This is indicated by the great number of studies on the new, only recently raised, problem of studying the regulatory mechanisms of cellular metabolism.

Brief summaries and exerpts from certain studies in this area are presented below.

Investigations in recent years have shown that living systems possess complex metabolic regulatory mechanisms which enable them to carry out their vital functions. On the basis of an investigation of these mechanisms and a survey of the results of other authors in this area, Neufach (1960) came to the conclusion that variation in the rates of enzymatic reactions is the only means of varyirg the intensity and the direction of metabolic processes at all levels from the molecular to that of the multicellular organism.

In turn, the rate of enzymatic reactions depends on the concentrations of substrates and coenzymes, the rate of the removal of the end products of the reaction, and the activity of the enzymes themselves. The mechanisms of metabolic regulation may be manifested in different ways: either in the form of competition of different enzymatic systems for the general substrates or the coenzymes are the object of competition. An example of the latter is the competition for ATP between the biosynthetic reactions and energy systems of a cell and so forth. A significant role in the regulation of cellular metabolism with its diverse enzymatic composition is played by the rate of the slowest reaction which, in the final analysis, determines the total rate of the entire process (Neufach, 1960).

Recently, a whole number of regulatory mechanisms operating on the feedback principle have been revealed (Engel'gardt, 1960; Kafiani, 1962 and others).

Those of the mechanisms which control protein synthesis are of particular interest. Most of the research dedicated to the investigation of enzyme formation has been carried out with the induced enzymes most suitable for this purpose. The investigation of the formation of other enzymes became possible only recently as a result of the discovery of certain metabolites capable of inhibiting the formation of the enzymes which participate in synthesis itself.

Umbarger (1961) demonstrated this form of regulation using the example of hexokinase, which is inhibited by glucoso-6-phosphate, one of the products of its reaction.

As opposed to this simple case of product inhibition, there is information concerning more complex inhibition according to the feedback principle. This is a matter of the action of an end product on a specific center in an enzyme molecule. Inhibition may be caused by factors which have structural affinity with the substrate, where they block the active center of the enzyme. Such inhibition leads to a change in the configuration of the enzyme and a disparity between the substrate molecule and the active center of the enzyme (J. Mono and F. Jacob, 1964).

The phenomena of enzyme repression and induction (Pardee, 1962) are among the mechanisms of the cellular regulation of the formation of new enzyme molecules. In the case of repression the end product of the reaction acts as an inhibitor which blocks the DNA sections which provide information for the synthesis of the enzyme protein. In the case of induction there is a concentration of the substrate which is accompanied by an intensified enzyme synthesis. The biological significance of regulatory mechanisms based on the repression and induction of enzymes is that they prevent the synthesis of an enzyme when there is no need for it at a given moment, and promote its formation when the need for it arises.

A comprehensive investigation of the chemical mechanism of the different fractions of subcellular particles by means of differential centrifuging showed that the regulation of the rates of enzymatic reactions may be determined not only by a deficit of the substrate or coenzymes, but also by spatial separation between the enzymes and the substrates or the coenzymes (Sikevits, 1962). A particularly important role is played by the impermeability of the intracellular membranes for certain substances. The hydrolase enzymes, most of which are found in lysosomes and have no connection with the rest of the cytoplasm because of impermeability

of the membranes, may serve as an example. The use of ATP, which is basically formed in the mitochondria, for energy-producing functions may be limited for the same reasons.

Refuting the contentions of a number of authors who explain the mechanism that regulates enzymatic reactions precisely by the spatial separation and impermeability of intracellular membranes, Neufach and his colleagues (1961, 1962) have put forth the fundamentally opposite view that these factors are of a dynamic nature. Neufach states that it is possible to explain the mechanism of the retardation or acceleration of a reaction only as being due to reversible changes in the structure and the permeability, and thus in the possibility for substances to migrate from one part of the cell to another. The reversibility of changes in the intracellular membranes depends on the ATP balance in the mitochondria. In the case of a high ATP level the membranes are impermeable. Their permeability increases as the ATP concentration decreases. Thus, the ATP concentration in a cell, the level of which serves as the source of information on the state of the energy exchange in the cell, is normalized. The mitochondrial membranes receive this information.

The data cited do not reflect all of the huge amount and the diversity of studies dedicated to this important general biological problem.

Many investigations of the energy regime of living organisms have established that most of the processes in which free energy is released always are accompanied by synthesis of ATP, a process during which energy absorption takes place. On the other hand, the synthetic processes which take place with an increase in free energy are accompanied by ATP splitting - a process which is accompanied by the liberation of free energy. Thus, ATP is a unique energy accumulator and serves as the connecting link between processes which supply and absorb energy.

High free hydrolysis energy is a characteristic of energy-rich phosphates of the ATP type. The special significance of these

compounds is that they serve as one of the basic energy sources necessary for numerous reactions of metabolic synthesis which takes place in living organisms.

The mechanism of the conversions of phosphorous compounds, many of which are both elements of biological structures and intermediate metabolic products, attracts the attention of many investigators. There is particular interest in the phosphorous transport reactions which are performed by ATP. This is the principal means of transporting energy from one chain of metabolic reactions into another.

On the basis of an investigation of the mechanisms of the enzymatic reactions of splitting certain organic phosphate compounds Kon (1961) adduced the way in which the splitting and restoration of ATP takes place.

The formation of ATP as the result of oxidative phosphorylation and glycolysis takes place through a series of reactions, the participants in which are orthophosphate and ADP. Although the use of ADP in biosynthetic reactions basically takes place by means of the splitting of ATP with the formation of ADP and orthophosphate, many such reactions proceed along a parallel path and lead to the formation of pyrophosphate and AMP. As a result of the action of two universally present enzymes, inorganic pyrophosphatase and adenylatkinase, pyrophosphate and AMP may be converted into orthophosphate and ADP. The hydrolysis of inorganic pyrophosphate is practically irreversible; it promotes biosynthetic reactions connected with the formation of pyrophosphate and replenishes the stock of inorganic orthophosphate. The cycle is thus completed and the inorganic phosphate again becomes available for the reaction of ATP formation which takes place in conjunction with energy supplying reactions.

In examining the question of the conservation of energy in a form accessible for use, George and Rutman (1962) stated: "...ATP or intermediate products obtained from it serve as the source of the moving force for a huge number of biosynthetic reactions". It is known that the free energy formed upon the oxidation of substrates

is used for ATP synthesis. There may be one or more predecessors of it, but ATP may be considered to be the first relatively stable intermediate product. It participates either directly in different reactions (orthophosphorylation, pyrophosphorylation, nucleotide transport), or in the formation of other cellular products in a large number of biosynthetic processes of the organism.

If the free energy of oxidation is to be retained to a significant degree, then it is necessary that intermediate phosphorous compounds possess particularly favorable free hydrolysis energy values. This follows from the fact that biosynthetic reactions may be divided into pairs of hydrolysis reactions, or one of these reactions serves in order to "set the other in motion". The greater the free hydrolysis energy of the first reaction is, the more completely does the second one proceed.

The selection of hydrolysis as the starting reaction for phosphates and intermediate products allied with them is dictated by purely practical considerations. If phosphates did not participate in any synthetic processes, they would degrade by means of hydrolytic splitting with the participation of an enzymatic catalyst, or even without it in view of the thermodynamic instability inherent in them.

The explanation of the role of phosphorous compounds in the transformation of the energy of biological oxidation into the energy of synthetic reactions and other processes which promote the life activity of the microbial cell is of general interest and has important significance for applied microbiology.

The numerous investigations and discoveries in this area during the last two decades have crystallized the generally accepted view that energy-rich phosphates play an exceptional role in carrying out the vital processes of macro- and microorganisms. A characteristic feature of macroergic phosphates is their exceptionally high free hydrolysis energy which exceeds the energy of ordinary phosphates by 3-4 times. This characteristic of macroergic phosphates enables them to be one of the basic energy sources in numerous reactions of

metabolic synthesis of the living organism.

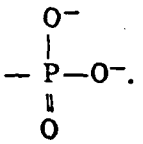
According to Pyul'man (1966), the entire set of metabolic transformations in the cell may be broken down into energy-releasing catabolic reactions characteristic of the processes of decomposition of complex compounds into simple ones, and anabolic reactions requiring external energy characteristic of the processes of the formation of complex structures from simple ones. As is known, living systems are subject to the first law of thermodynamics; therefore, it is natural that the energy source for the anabolic processes should be the catabolic reactions. This is the basis for the most important concept - energy linkage - in biochemical energetics. According to this concept, energy released in the processes of the decomposition of a substance may be used in synthesis reactions.

As Pyul'man (1960) indicates, quantitatively the linkage concept is connected with the concept of the variation of the amount of free energy (ΔF) of a system during the course of a reaction. Free energy, in the framework of chemical concepts, is energy capable of performing a certain reaction. Reactions accompanied by a decrease in free energy ($\Delta F<0$) are called exergonic, and those accompanied by an increase ($\Delta F>0$) are called endergonic. From the thermodynamic point of view, the requirement that the energy released during an exergonic reaction exceed the energy required during an endergonic reaction is the condition which must be met in order for an exergonic reaction to make possible the onset of an endergonic one. From the chemical point of view, the accomplishment of a linkage reaction requires that the energy transfer from one reaction to another take place through a reagent which participates in both reactions. In the most general form, this process comes down to the fact that one of the reagents which comes from a chosen donor takes on a radical which changes its free energy in the corresponding direction. In the huge majority of metabolic reactions the phosphoryl group R serves as this radical:

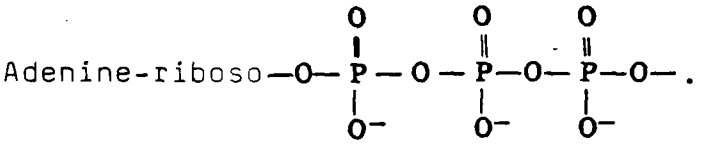
$$R = -\overset{\displaystyle OH}{\overset{|}{\underset{\displaystyle O}{\overset{\|}{P}}}}-OH,$$

which in the case of physiological pH values exists, as a rule, in

the form:



Adenosine triphosphate, ATP, the hydrolysis of which to adenosine diphosphate and inorganic phosphorous pH is accompanied by a change in free energy to ΔF=8 kcal/mole, is the chief "supplier" of this radical.



Energy-rich phosphates belong to the category of conjugate molecules. This is confirmed by the fact that the mobile electrons of the phosphoryl radical are connected either with mobile electrons of another phosphoryl radical (in the case of pyrophosphate), or with the electrons of other groups possessing mobile electrons. The phosphoryl group of energy-"poor" phosphates: AMP, glucoso-6 phosphate and others are connected with a saturated carbon atom. The hydrolysis of ATP is a reaction which may take place under the influence of certain enzymes. It is known that in biological energy convertors, such as all living systems are, there are mutual conversions chiefly of chemical energy on the one hand, to heat, mechanical, electrical, and elastic (surface) energy, on the other. There is no special biological form of energy. Therefore, both laws of thermodynamics, the first and the second, are applicable to living systems. As far as the role of entropy in living systems is concerned, the majority of investigators limit it. They assume that the direction of metabolic chemical reactions in living systems is regulated by biological laws, the necessity for the best adaptation of a given type of organism to the conditions of its existence and the action of natural selection (Alekseyev, 1966).

The free energy of the oxidation of food products (carbohydrates, fats, and proteins) plays the basic role in the life of all organisms, including microorganisms. It has been found that, in the case of the oxidation or fermentation of sugar, around 50% of the

energy contained in it accumulates in "energy-rich" phosphates - ATP or in the bonds of macroergic substances. The synthesis of ATP from ADP and free orthophosphate takes place continuously. Energy and orthophosphate are released in the case of the decomposition of ATP without the addition of oxygen. Thus, Konovalov (1967) indicates that two diametrically opposed processes: the synthesis and the decomposition of ATP, which are very favorable for yeasts in an energetic regard, take place during a closed cycle.

Brintzinger (1961), Russel and Wyard (1961), and Skulachev (1965) and others allow for the possibility of the existence of an internal reaction between the component parts of ATP and ADP, expressed, evidently, by the existence of internal transport of electrons in the molecule. It is assumed, for example, that ADP must be considered not only as an energy accumulator of chemical bonds, but also as a transformer of this energy, liberated during an electron transport. At the present time, the assumption that the adenine radical of the ADP molecule transforms the energy of phosphate bonds, that is, that adenine must be considered to be an energy transformer, is shared by a whole number of investigators. Engel'gardt, Shapot and Lenindzher, Gonzales, and Shuster (1963), and others (cited by Konovalov [1967]) indicate that ADP not only plays an important role in energy conversions and connects exergonic reactions with endergonic ones, but also as a universal energy donor, creates the general cellular energy reserves, provides for the redistribution thereof for satisfying different biosynthetic processes, and participates in the regulation of functional changes in these cells.

Krebs, Kornberg and others (cited by Konovalov, 1967) distinguish three biochemical phases of energetic exchange. An analysis of these phases shows that in an energetic regard the role of the first and second phases is insignificant, and the third - the tricarboxylic acid cycle - is the basic phase not only of the energetic but also of the growth metabolism of yeasts. Two-thirds of all energy is liberated in this phase. Thus, it is the most effective from the energetic point of view. The formation of ATP and other phosphorous compounds in this phase predominates over

their decomposition. The rate of formation of cellular matter and cell division depend on the amount of ATP. Cell vitality weakens with the cessation or attenuation of ATP formation. It has been found that the coenzymatic role of ATP during fermentation and respiration reduces to the fact that ATP, having given off labile phosphate to the substrate, turns into a lower degree of phosphorylation, and then is phosphorylated into ATP. Moreover, 47% of all energy which is liberated during the oxidation of glucose is accumulated in the form of macroergic ATP compounds.

In the case of the anaerobic decomposition of glucose two ATP molecules are formed, in the case of the oxidation of glucose - 32 molecules, or 16 times more. Comparing the anaerobic process of glucose decomposition and the oxidation thereof in aerobic conditions, it must be said that the aerobic process, from the biological point of view, is more economical and more effective from the energetic and chemical points of view.

In studying the dynamics of the accumulation and expenditure of polyphosphates by microorganisms, the following conclusions were reached: in the lag phase, preparation for intensive reproduction is accompanied by the transformation of soluble polyphosphates into physiologically more active polyphosphates insoluble in acid. In the first half of the logarithmic phase, as a result of redistribution between cells, especially as a result of expenditure on synthetic processes, the amount of polyphosphate insoluble in acid per cell sharply decreases. The end of the logarithmic and the beginning of the stationary phases are characterized by an increase in the less physiologically active acid soluble polyphosphates.

Cytochemical and biochemical methods have revealed that, in conditions unfavorable for reproduction, and in the case of the artificial retardation thereof, a significant amount of polyphosphates insoluble in acid accumulates in these cells. In individual cases the energy of polyphosphates may be used by cells even without the participation of ATP. However, the greatest expenditure of polyphosphates for synthetic processes always is connected with the

maximum ADP and ATP contents of the system. If each P-O-P bond in a polyphosphate molecule is macroergic, then they must be not only gigantic accumulators of energy and phosphorous, but also transporters thereof from cell to cell.

Verbina indicates that polyphosphates maintain constancy in the relation between ADP and ATP in the cell and together provide for optimal conditions for many metabolic processes in the cell.

Numerous investigations show that the energy exchange in a microbial cell is subject to the general principles of the energetic organization of higher multicellular organisms. Like the latter, biological oxidation in the microbial cell also is connected to the synthesis of energy-rich phosphorous compounds. Being accumulators of the energy of biological oxidations, macroergic phosphorous compounds transform it into the energy of biosynthetic reactions and other processes of the microbial metabolism. A whole series of factors, for example, the activity of the phosphatases which hydrolyze ATP, the concentration of mineral phosphorous, the presence or absence of acceptors of macroergic phosphate groupings, and so forth, may be regulators of the intensity and direction of the metabolic processes in the cell. In this connection, the transformations of phosphorous compounds with a complete base may be included in the general system of the complex regulatory mechanism of the microbial cell. It must be emphasized that the use of microorganisms as objects of investigations had great significance in the success achieved in recent years in explaining the role of macroergic phosphorous compounds. This is easy to understand if we consider the diverse chemical specificity of microorganisms.

Thus, recent discoveries have confirmed the opinions of those classical writers in microbiology, who, at the dawn of our era of grandiose biochemical discoveries, had high regard for the activity of microorganisms as specific, as Vinogradskiy calls them, "embodied" chemical reagents.

It is known that the activity of microorganisms in nature takes place chiefly in conditions of the interaction of different species.

Activity in isolation (a pure culture) is an uncommon phenomenon in nature. Imitating the natural collaboration of microorganisms in experimental conditions, Nentskiy (cited by Silishchenskaya, 1956), at the end of the last century, first showed the advantages of a mixed culture over a pure one in the case of fermentation and acid formation of a number of microorganisms. He proved that mixed cultures of R. paralactici and Clostr. chavoei form butanol from glucose although the two collaborators do not produce alcohol in isolation. In the case of this type of microbial interrelationship, called synergism, not only may the basic functions of the microbial participants of the mixed culture be strengthened, but also new functions not characteristic of their individual growth also may arise. In addition to synergism metabiosis, the essence of which is that certain macroorganisms by the products of their metabolism supply material for the nutrition and subsequent development of other microorganisms, is one of the most common, practically important, types of microbial interrelationship.

The literature dedicated to this problem contains numerous results of experimental studies illustrating the advantages of synergitic cultures over pure ones and the effectiveness of using metabiotic interrelationships of microorganisms in practice. Individual excerpts from these studies are presented below.

Castellani (1925) put together mixed cultures of a large number of pairs of microorganisms, which on different sugars produced gas only in a mixed culture; in isolation these cultures did not form gas. Bogdanov (1936) succeeded in significantly accelerating the ripening of cheeses by using a mixed culture of lactic acid bacteria.

Imshenetskiy (1939) obtained three times more alcohol with mixed cultures of cellulose bacteria than with pure cultures of the same bacteria.

Gibshman (1945) noted an increased formation of acids and more rapid curdling of milk in the case of the joint cultivation of lactic acid bacteria with proteolytic bacteria than with pure

cultures of lactic acid bacteria alone.

Silishchenskaya (1956) studied a natural synergitic culture of homo- and heterofermentative lactic acid bacteria and found an increase in the rate of acid formation of four times in comparison with pure cultures of its components. In addition, heterofermentative lactic acid bacteria in a mixed culture lost the ability to form by-products and formed only lactic acid.

Prescott and Dane (1952) and others demonstrated the advantages of mixed cultures in the formation of lactic acid and the production of certain products of fermentation instead of others.

In bakeries in Kazakhstan, Shamis, Masheyeva and Tukibayeva (1963) introduced a mixed culture composed of four strains of the yeast Sacch. cerevisiae, which at different stages of dough formation mutually supplemented one another, improving the production techniques. The same authors put together a mixed culture of the yeasts Sacch. cerevisiae and Schizosacch. pombe, having high enzymatic activity; the dextrinase activity of the latter helped prevent the dough from falling when being spread out.

Imitating the metabiotic interrelations of cellulose bacteria and Azotobacteria in the soil, Shamis et al. (1966) showed that it was possible to cultivate Azotobacter on the products of the chemical hydrolysis of a cell - hydrolyzates. In addition, in the case of joint and successive cultivation of Azotobacter and food yeasts on hydrolyzate media the yeast yield was increased and the organic substances of these media were more fully utilized.

Berezina and Dubitskaya* studied the conditions of joint and successive growing of food yeasts and lactic acid bacteria utilizing pentoses and obtained a mixed culture of these microorganisms with a high nitrogen content and an altered quantitative composition of amino acids including essential ones.

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*See the article by these authors in this volume.

In our brief survey of the literature we found only an insignificant number of studies dealing primarily with applied microbiology. However, synergism and metabiosis are characteristic of microbiological processes and other areas of microbiological science.

The investigation of the question of the possible utilization of bacteria which actively split organophosphates in order to increase the yield of food yeasts is one aspect of the general direction of studies in imitation of natural interrelationships of microorganisms being carried out in the Laboratory of Microbial Protein Synthesis of our institute during recent years.

The use of a mixed culture in producing food yeasts and the inclusion in such a culture of precisely those bacteria which split organophosphates is justified:

(a) by factual data in the literature on the advantage of a mixed culture of microbes over a pure culture;

(b) by the assumption, on the basis of the imitation principle, that the bacteria which split organophosphates in the soil, as a result of their high phosphatase activity, may be used in a mixed culture with yeasts in order to accelerate the conversion (mobility) of macroergic phosphorous compounds and thus intensify biosynthetic processes in the yeast cell;

(c) by the assumption that the active phosphatase of bacteria that split organophosphates may be a factor in the propelling of energetic reactions (Engel'gardt, 1940) for the activation of yeast phosphatase. In addition, the possibility that the yeast may borrow the bacterial phosphatase coenzyme is not excluded.

The short experimental part of this article presents preliminary results of an experiment in using bacteria which actively split organophosphates (Bac. megaterium), in joint and successive cultures with yeasts.

Technique and Object of Investigation

The food yeasts Candida tropicalis and Bac. megaterium (bacteria which split organophosphates) were grown jointly and successively (bacteria-yeasts) in a synthetic medium put together on the basis of the ingredients of synthetic media used in the separate growing of these microorganisms. The composition of the mixed synthetic medium for yeasts and bacteria was usedas follows: glucose - 10 g; $(NH_4)_2SO_4$ - 2 g; $MgSO_4$ - 0.2 g; KCl - 0.2 g; NaCl - 0.3 g; microelements - 1 ml; yeast autolyzate - 10 g; organophosphate source - 0.5 g; tap water - 1 l; pH=6.5. The innoculated yeast culture was grown on a synthetic medium for yeast and removed after 24 h, and the innoculated bacteria culture was grown on a Menkina medium and removed after 5 days. We took 10 ml of a yeast suspension and 20 ml of a bacterial suspension for innoculating the mixed synthetic medium. The separate growing of yeast and bacteria on the mixed synthetic medium served as the control.

The following served as phosphorous sources: nucleic acid, sodium ATP, and orthophosphate (control). The microorganisms were grown in laboratory rockers moving at a rate of 150-180 r/min, at t=28-30°. The yield and quality of the yeast were evaluated according to the criteria presented in the table.

An analysis of the data presented in the table showed the following. In the case of the joint cultivation of yeast and bacteria which split organophosphates, the biomass and nitrogen content of the yeast (criteria characterizing the intensity of biosynthetic processes) exceed the values obtained in the case of the separate growing of yeast. In the case of using ATP as a phosphorous source, this increase reaches 20%. The yeast themselves also utilize organophosphates well.

In the case of a combined culture, the ATP content of the yeast, irrespective of the phosphorous source, exceeds the ATP content in the case of a separate culture.

A higher total nitrogen content is observed in the case of the successive culturing of bacteria and yeast when ATP serves as the phosphorous source.

Yeast yield criteria and nitrogen and phosphorous content of yeast in different growing conditions.

(a) Характер культивирования	(b) Источник фосфорного питания	(c) Сухая биомасса, г	(d) Общее кол-во азота в дрожжах, г	(e) Общий азот, %	(f) P, мг на 0,1 г культуры: (g) лабильный P_7	(h) стабильный	минеральный	(j) общий	(k) АТФ, мг
(l) Совместное культивирование дрожжей *Candida tropicalis* и бактерий *Bac. megaterium*, расщепляющих органофосфаты	АТФ (o)	0,645	0,452	7,0	38	25	19	63	10,3
	Нуклеиновая к-та (p)	0,590	0,413	7,0	44	26	24	70	13,0
	Ортофосфат (q)	0,619	0,365	5,9	44,3	16,7	26	61	13,5
(m) Последовательное культивирование бактерий *Bac. megaterium*, расщепляющих органофосфаты, и дрожжей *Candida tropicalis*	АТФ (o)	0,464	0,450	9,7	22	28	26	50	8,5
	Нуклеиновая к-та (p)	0,536	0,392	7,31	61,3	—	39	49	19 0
	Ортофосфат (q)	0,381	0,248	6,5	56,8	5,2	33	62	17,3
(n) Раздельное культивирование дрожжей *Candida tropicalis*	АТФ (o)	0,517	0,384	7,42	21 9	60,1	26	82	8,5
	Нуклеиновая к-та (p)	0,509	0,327	6,42	30,6	16,4	19	47	9,5
	Ортофосфат (q)	0,527	0,316	6,0	45,6	24,4	27,5	70	8,9

Key: (a) Nature of culture; (b) Phosphorous source; (c) Dry biomass, g; (d) Total amount of nitrogen in yeast, g; (e) Total nitrogen, %; (f) P, mg per 0.1 g of culture; (g) Lability; (h) Stability; (i) Mineral; (j) Total; (k) ATP, μg; (l) Joint culture of Candida tropicalis yeast and Bacterium megaterium which splits organophosphates; (m) Successive culture of Bac. megaterium which splits organophosphates and Candida tropicalis yeast; (n) Separate culture of Candida tropicalis yeast; (o) ATP; (p) nucleic acid; (q) orthophosphate.

As is obvious from the table, there is more labile phosphorous (P_7), basically consisting of physiologically active polyphosphates, in the case of a joint culture of bacteria and yeast than in the case of growing yeast alone. However, there is also a high labile phosphorous content in all variants of the experiment where orthophosphate served as the phosphorous source.

Conclusion

This article reflects the theme of one of the areas of investigation of the Laboratory of Microbial Protein Synthesis, where we are studying the factors which accelerate the conversion (mobility) of

macroergic and other phosphorous compounds. The purpose of these studies is to increase the energy supply of the metabolic synthesis reactions connected with the yield and quality of food yeast.

The article presents data cited in the literature where the ideas of the American investigator John Wait are examined. On the basis of theoretical calculations the author came to the conclusion that no less than one-third of the sugar carbon, irrespective of the yeast yield, is split off in the form of carbon dioxide. However, Wait did not take account of the fact that, as a result of the heterotrophic assimilation of carbon dioxide by yeast for the biosynthesis of different compounds, the amount of carbon dioxide split off will always be less.

Stating from Wait's concept, Professor Malkov sees the reason for the incomplete yeast yield to be precisely the fact that yeast "uneconomically" expend more than one-third of the carbon in respiration, therefore leaving less than two-thirds of the carbon for biosynthesis. On this basis Malkov conducted a number of investigations where he strove to increase the yeast yield by means of the partial inhibition of respiration with different inhibitors. This article presents the results of one such experimental study. However, the causes mentioned by Malkov, thanks to which he achieved an increase of 15% in the yeast yield, do not agree with the data in the literature. For example, it is known that Meyrhof, in studying the "economizing", according to his expression, effect of aeration on carbon expenditures experimentally proved that aeration sharply reduces these expenditures per unit of yeast yield from the genus Torula. It is also known that strangulation of the respiration process causes attenuation of biological processes.

However, Professor Malkov, the author of the idea of the partial inhibition of respiration in order to save carbon, mentions another factor which may be the cause of intensification of the biosynthetic processes of the yeasts he investigated. We are speaking here about the doubled amount of labile phosphorous P_7 obtained in yeast as a result of placing it in concentrated orthophosphate.

The increased P_7 content of yeast subjected to any complex influence is in agreement with the data in the literature. In our investigations the increased labile phosphorous content is connected with the biosynthetic activity of the yeast.

The article correlates many studies which show the very important role of ATP and polyphosphates as energy accumulators and transporters, and presents data from the literature and preliminary results of our own investigations which indicate the exceptional effectiveness of making practical use of the phenomena of microbial synergism and metabiosis. Bacteria which split orthophosphates were studied on the basis of these results. There is also significant interest in the phosphatase activity of food yeast using organophosphates as a phosphorous source. Research in this area is only just beginning.

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